

Do vaginal lactobacilli prevent preterm labour?

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Objective. To determine the prevalence of *Lactobacillus* spp. in vaginal flora during pregnancy and to assess the protective effects of lactobacilli against preterm labour.

Design. Cross-sectional analysis of *Lactobacillus* spp. in the vaginal flora of the pregnant coloured population of the Western Cape.

Participants. A total of 480 consecutive pregnant women, aged 13 - 48 years, seen at their first visit to the Tygerberg Hospital antenatal clinic.

Main outcome measures. Preterm labour, i.e. before 34 and 37 weeks' gestation, premature rupture of membranes, intra-uterine growth retardation and perinatal deaths.

Results. A total of 163 patients had negative cultures and 317 positive cultures for lactobacilli, aerobes or both. Delivery before 37 weeks occurred in 18% and 20% of the two groups, respectively. *Lactobacillus* only was cultured from 116 patients and *Lactobacillus* and/or other aerobes from 201 patients. Preterm labour occurred in 20% of the first group and in 19% of the second group. The perinatal outcome in patients from whom lactobacilli only were cultivated did not differ from patients from whom other aerobes and lactobacilli or other aerobes only were cultured.

Conclusion. In patients at high risk for preterm labour, the presence of lactobacilli in the vagina does not seem to play a protective role.

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At Tygerberg Hospital 20% of babies are born before 37 weeks' gestation and 7% before 34 weeks (obstetric database — Tygerberg Hospital). Therefore, it is not surprising that preterm labour is the main cause of perinatal mortality.¹ According to recent information, infection and particularly vaginal bacteriosis play a major part in the pathophysiology of preterm labour.²⁻⁶ On the other hand, normal vaginal flora have important protective effects and its disruption may predispose to disease.⁷ It is generally assumed that the vaginal lactobacilli are the most important organisms responsible for this protective effect.⁸⁻¹⁰

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It was therefore decided to determine the effect of the vaginal lactobacilli on the prevalence of preterm labour. As the neonatal death rate is much higher among babies born before 34 weeks' gestation than between 34 and 37 weeks, this group was analysed separately.

Patients and methods

Vaginal swabs were taken from 480 consecutive pregnant women at the first antenatal visit. After the cervix had been exposed with a sterile speculum, swabs were taken from the posterior fornix. Any contact with the external genitalia and other potential sources of contamination was carefully avoided. No transfer media were used and cultures for *Lactobacillus* and other aerobes were inoculated into suitable culture media within 2 - 4 hours after the swabs had been taken. For the isolation of lactobacilli, De Man, Rogosa and Sharpe (MRS) plates were inoculated with the swabs and incubated anaerobically at 37°C for 48 hours. Cultures obtained were then identified to species level through a series of standard identification tests described by Hammes *et al.*¹¹ These were as follows: Gram stain, catalase test, gas production from glucose, ammonia production from arginine, growth at 15°C and 45°C, and appropriate carbohydrate fermentation tests.

The obstetric history obtained from the patients included information about previous premature rupture of the membranes and preterm delivery. Patients were followed up prospectively until after delivery. Gestational age was calculated either on the basis of an ultrasound examination between 16 and 24 weeks or the patient's last menstrual period.

At the end of the study, microbiological findings were compared with the perinatal outcome. Categorical data were analysed by means of the χ^2 test or, when the numbers were small, Fisher's exact test. Continuous variables were compared with Student's *t*-test when data were normally distributed and the Mann-Whitney U-test for non-normal distributions. A significant difference was considered present if $P < 0.05$.

Results

The mean age of the mothers was 26.4 (SD 6.3) years. Fifteen per cent were younger than 20 years. The median parity and gravidity were 1 and 2, respectively. One hundred and fifty-three patients (31%) were primigravidas and 45 (9.4%) had had more than four previous pregnancies. Fifty-six mothers had previously had preterm deliveries while 2 women had experienced previous premature rupture of the membranes. The mean gestational age was 37.6 weeks (SD 3.7) and the mean birth weight was 2 898 g (SD 755). Fifty-two babies were small for gestational age. Nine mothers experienced premature rupture of the membranes and 13 developed fetal distress.

Positive cultures could be obtained in 317 (66%) of the 480 patients. Of these, 116 were of lactobacilli alone, 62 of lactobacilli and other aerobic organisms and 139 of other organisms only (Table I). In 82 lactobacilli cultures, organisms could be identified at species level. They

belonged to six different *Lactobacillus* spp., viz. 27 *L. plantarum* isolates (33%), 19 *L. acidophilus* isolates (23%), 19 *L. brevis* isolates (23%), 14 *L. fermentum* isolates (17%), 2 *L. casei* isolates (2%) and 1 *L. gasseri* isolate (1%). No *Lactobacillus* or other bacterial growth was seen in 163 (34%) patients. It should be stressed, however, that no cultures for anaerobes were performed.

Table 1. Comparison of clinical data in patients with different vaginal cultures

	A	B	C	D	N
Bacterial culture	163 (34%)	116 (24%)	62 (13%)	139 (29%)	480 (100%)
Previous preterm delivery	21 (13%)	19 (16%)	5 (8%)	11 (8%)	56 (12%)
Previous PROM	1	0	1	0	2
Mean gestational age	37.6	37.2	38.3	37.7	-
< 37 wks	29 (18%)	26 (22%)	12 (19%)	26 (19%)	93 (19%)
< 34 wks	14 (9%)	11 (9%)	5 (8%)	10 (7%)	40 (8%)
PROM	2	1	2	3	8
IUGR	18 (11%)	12 (10%)	10 (16%)	13 (9%)	53 (11%)
Fetal distress	2	2	2	7	13
Apgar score (median)	9	9	9	9	9
Mean birth weight (g)	2 902	2 835	2 936	2 930	-
Perinatal deaths	11 (7%)	10 (9%)	4 (6%)	4 (3%)	29 (6%)

A = negative; B = lactobacilli only; C = lactobacilli and other aerobic organisms; D = only aerobic organisms; PROM = pre-labour rupture of membranes; IUGR = intra-uterine growth retardation.

No significant difference could be found between A v. B + C + D, A v. B, B v. C + D, B v. C, C v. D.

The rate of previous preterm labour in the four groups ranged between 8% and 16%. Eighteen per cent to 22% of mothers delivered before 37 weeks and 7 - 9% before 34 weeks. Mean birth weights ranged between 2 835 g and 2 936 g. Mothers with negative cultures did not differ significantly from those in whom different cultures were found (Table 1, A v. B + C + D). Mothers with negative cultures did not differ from those in whom lactobacilli only were cultured (A v. B). The lactobacilli-only group also did not differ from the groups in whom lactobacilli and other organisms (B v. C) and other organisms only were cultured (B v. C + D). Lastly, the lactobacilli and other organisms group did not differ from the other organisms-only group (C v. D).

Discussion

It has been suggested that lactobacilli limit the growth of pathogenic bacteria and thus protect against infection.^{8,12,13} However, although vaginal colonisation with *Lactobacillus* may be protective against infection, we are not aware of

studies where the introduction of exogenous lactobacilli into the vagina actually improved perinatal outcome.

Our finding that the presence or absence of *Lactobacillus* in vaginal cultures did not have any effect on the duration of pregnancy or the prevalence of preterm delivery was very unexpected as it is generally accepted that lactobacilli play a protective role. A previous study at this institution indicated that certain vaginal infections had an adverse effect on the outcome of pregnancy.¹⁴ Preterm labour occurred in 47% of patients in whom *Chlamydia trachomatis* had been identified in the vagina, compared with 17% of those patients in whom no *C. trachomatis* could be identified.

The same study demonstrated that significantly more patients in whom *Candida albicans* had been identified experienced preterm labour than did patients in whom no *C. albicans* could be demonstrated. As *C. albicans* is known to grow better when the vaginal pH is more acidic, it was surprising that these patients experienced more preterm deliveries. In another study at this hospital, it was demonstrated that the administration of ampicillin and metronidazole to patients in preterm labour delayed eventual delivery; this suggests the presence of an underlying infection.¹⁵

The association between preterm labour and different genital infections has been confirmed in many other studies.¹⁶⁻²⁰ Bacterial vaginosis has been identified as a major risk factor for preterm labour when it occurs in the second trimester of pregnancy, but not when diagnosed at 28 - 32 weeks.² Although it is still uncertain by which means infection causes preterm labour, it is known that the normal vaginal flora changes from being predominantly *Lactobacillus* to predominantly *Bacteroides* spp., *Gardnerella vaginalis* and *Mycoplasma hominis*.

Our finding that the presence of lactobacilli in the vagina of pregnant mothers has no effect on the duration of pregnancy or the prevalence of preterm labour could be due to the lack of protection of specific *Lactobacillus* species, a low concentration of lactobacilli, or perhaps more probably an overwhelming bacterial vaginosis where the protective effects of lactobacilli were lost. The protective effects of vaginal flora are therefore more complex than the mere presence or absence of lactobacilli.

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